

I have not been able to examine the large number of forms included by Saccardo under *Monilia*. Of these it is probable that many have no real affinity with that above discussed; while others may be closely related. Such appears to be the case with a rather common form which attacks the immature fruits of *Prunus serotina* and related species, forming delicate white tufts, with spores very like, but somewhat smaller than those of *M. fructigena*. This is probably the plant called by Saccardo²⁶ *Monilia Peckiana*, var. *angustior*. The few cultures I have been able to make have yielded only the common spore-chains, but the form will probably repay further investigation as to its pleomorphism and its affinities.

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EXPLANATION OF PLATE VII.

- Fig. 1. Two *Monilia* hyphæ passing into branching spore-chains. $\times 540$.
 Fig. 2. The end of a spore-chain, showing the origin of branching from two terminal angles of the terminal cell. $\times 540$.
 Fig. 3. Bits of resting mycelium from the flesh of a "mummied" plum. $\times 540$.
 Fig. 4. Resting cells, "gemmae," from the same source. $\times 540$.
 Fig. 5. Hyphæ from *Monilia* spores, with oblong bodies developed within the gelatine. *a*, $\times 540$. *b*, $\times 940$.
 Fig. 6. Three *Monilia* spores producing germ-tubes with sterigmata and microconidia. *a*, germinating conidia. $\times 940$.
 Fig. 7. Chains of microconidia from *Monilia* hypha, in culture. $\times 540$.
 Fig. 8. Microconidia germinating on nutrient gelatine. *b*, after one day, $\times 940$. *a*, after 2 days, $\times 540$.

Non-parasitic bacteria in vegetable tissue.¹

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It has been ascertained that the tissues of the animal body in their normal healthy state are perfectly free from bacteria but the evidence that the same is true in regard to vegetable life is not so unanimous. The subject has received considerable attention at the hands of bacteriologists and while the majority of observers do not admit that bacteria are to be found in vegetable tissue in a healthy state, some proof has been brought forward to support such a conclusion¹.

In a course of experiments, which have been carried on for

²⁶Sylloge Fungorum, iv, 34.

¹Read at the Rochester meeting of the A. A. A. S., August, 1892.

some time in another connection, I have noted a number of points that seem to have a direct bearing on this question and afford a possible explanation for the contradictory evidence previously at hand.

The effect of the inoculation of various saprophytic as well as facultative parasitic species of bacteria into the tissue of different plants was studied in order to determine first, the effect produced on the plant, if any, and second, the reciprocal effect on the micro-organism.

Caulescent plants,² such as Geranium, were selected and into these were injected various forms of micro-organisms, after washing the stem with sterile water to rid it as far as possible of the bacteria on its surface. The minute opening in the stem caused by the inoculating needle was closed by sterile vaseline, so as to prevent the entrance of foreign organisms. In this way different species of bacteria were introduced into the interior of rapidly growing healthy plants, and after varying periods of incubation, their effect was ascertained. With certain saprophytic forms such as *B. megaterium*, *B. lactis aërogenes*, *B. butyricus*, etc., as well as those species pathogenic for animals like *B. anthracis*, *B. typhosus*, *B. diphtheriae columbarum*, no macroscopic change could be detected in the tissue of the host when it was in a healthy growing condition. Of course those forms that are pathogenic for plants, such as pear blight, did affect their respective hosts when inoculated into susceptible plants, but when species not closely related to their normal hosts were used, no macroscopical change could be noticed in the condition of the plant. A microscopical examination of the tissues also failed to reveal any pathological conditions brought about by the injection of the micro-organisms.

So much for the effect of the micro-organism on the plant. The effect of the host upon the micro-organism was, however, greatly different. This was determined by means of culture experiments. The plant tissue into which the bacteria had been injected was taken after varying periods of incubation and the cortical ring of tissue removed by sterile scalpels, after which quite thin transverse sections of the remaining core of tissue were sectioned, also under aseptic precautions. These were then seeded in melted gelatine tubes

²Fazio: *Revista Intern. d'Igiene* (1890); Galippe, and others.

and roll cultures made therefrom. The liquefied gelatine penetrates the section and the bacteria present are able to grow. Not only were cultures made in this way from the tissue taken at the point of inoculation but sections were also taken at varying intervals both above and below the point of introduction. Special care was taken in the use of germ-free instruments, so that there was no possibility of transferring bacteria from one section to another.

The results obtained in this way were quite various. Nearly all of the species pathogenic for the animal body, as *B. anthracis*, *B. diphtheriae*, *B. cholerae gallinarum*, *Micrococcus tetragenus*, *M. cereus flavus*, *Staphylococcus epidermis albus* and *St. pyogenes aureus*, were killed out in the plant tissues after a lapse of a few days. One notable exception was, however, observed in the case of *B. pyocyaneus*. This germ was able to live in healthy plants of different species like *Geranium*, *Penthorum* and *Begonia* for 50 to 70 days or more and even able to spread throughout the plant tissue in an upward direction to a distance of 50 to 80^{mm}. By far the greater number of the parasitic bacteria, however, were unable to survive in the plant.

With saprophytic bacteria, the case was usually different. *B. prodigiosus*, *B. butyricus*, *B. luteus*, *B. coli communis*, *B. fluorescens*, and the lactic acid germ were all to be found in large numbers after varying periods of incubation ranging from 20 to 50 days, not only at the point of inoculation but in gradually lessening numbers for some distance up the stem.

Other species like *B. megaterium* and *B. lactis aërogenes* were to be detected in the tissue after 40 days but only in small numbers and *only* at point of inoculation.

Now these results indicate that bacteria, saprophytes at least, can live in the tissue of plants for a considerable length of time, and the fact that they are able not only to exist at the point of introduction but to spread throughout the tissue to a limited extent raises the question as to whether they are not really able to grow, to a limited extent at least, if they once gain access to the less resistant tissues of the inside of the plant.

On this point, enough data have not yet been gathered to say definitely, but these observations are presented with ref-

erence to the first question raised, as to the presence of bacteria in normal healthy tissues.

It has been shown that certain bacteria *can* live in plant tissues for a long time, if they are once allowed an entrance (as by artificial inoculation). What happens by artificial inoculation can under certain circumstances also take place in a state of nature. If by any means, a plant is wounded in any way, bacteria can enter from the air, and if the tissues are succulent enough, they can at least live for considerable time. Bacteria introduced in this way as wound parasites would come to be enclosed in the tissue by the healing over of the wounded surface, and thus error might arise as to their origin even if the experiments were carried out on the most rigid of bacteriological principles. A minute puncture would suffice to allow them to enter, as was to be seen when a sterile platinum needle was thrust into the tissue of a stem and then the wound closed by vaseline as before. Cultures from the pith parenchyma showed bacteria that were also to be found on the epidermis of the plant.

In connection with the above results, I have made numerous attempts to isolate micro-organisms from different forms of vegetable tissue, where I first made sure that there were no wounds, but in no case have I been able to isolate them when the conditions of the experiments were faultless. The appearance of a single colony or so in the cultures occasionally, is due to the inevitable exposure at the time of preparation of cultures. The danger of external contamination is much less where stems of fairly good size are used instead of such subterranean organs as rhizomes. Bacteria are present in the superficial soil layers in myriads and it is easy to see that by some slight abrasion or puncture of the epidermis they can gain access to the inner tissues of the plant and here live for a considerable length of time.

The evident conclusion from these results is that vegetable like animal tissue is normally free from micro-organisms, but that in healthy plant tissue many species of bacteria are able to exist for a not inconsiderable length of time. This is true with the most healthy growing plants, and where the vitality of the plant is weakened to any great extent, the micro-organism is much more able to sustain itself in the struggle for existence.

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